Attraction of Males by Virgin Females of the Mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae)

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Environ. Entomol. 30(2): 339–345 (2001)

ABSTRACT The mealybug *Maconellicoccus hirsutus* (Green) has extended its range throughout the Caribbean region since it was first detected in Grenada in 1994, and has recently been detected in Southern California, Mexico, and Central America. Laboratory and field experiments using virgin females were conducted on St. Croix, U.S. Virgin Islands, to determine if females attract males with pheromones. Virgin females isolated in gelatin capsules attracted on average one male to each capsule over a period of 18 h in the laboratory compared with gelatin capsules without females. Adhesive traps baited with virgin females and placed on hibiscus, *Hibiscus rosa-sinensis* L., in the field, captured more males at all three study localities on St. Croix than did unbaited traps. Virgin females attracted more males than controls at 0–10 m from infested hibiscus, but were capable of attracting males at 50 m distance from an infestation. The attractiveness of virgin females to flying males strongly suggests the involvement of a female-produced sex pheromone. Isolation and synthesis of such a sex pheromone would provide a valuable tool for population monitoring and control of this invasive pest.

KEY WORDS *Maconellicoccus hirsutus*, sex pheromones, invasive pests, quarantine, Caribbean region

THE MEALYBUG *Maconellicoccus hirsutus* (Green) was first detected in the Caribbean on the island of Grenada in 1993. Since then, it has spread north through the Lesser Antilles to infest the U.S. and British Virgin Islands and Puerto Rico, and south to northeastern South America (Guyana), and west to Central America (Pollard 1998, Sagarra and Peterkin 1999). *M. hirsutus* was detected in September 1999 in southern California, and subsequently Mexico and Belize (D.E.M., unpublished data). In the Caribbean, *M. hirsutus* prefers to feed on ornamental hibiscus (*Hibiscus rosa-sinensis* L.), blue mahoe (*Hibiscus elatus* L.), sorrel (*Hibiscus sabdariffa* L.), soursop (*Anona muricata* L.), sugar apple (*Anona squamosa* L.), and saman trees (*Samanea saman* L.) (Persad 1995, Williams 1996); it has a reported host range of >125 plant species worldwide (Ghose 1972, Williams 1986, Mani 1989). *M. hirsutus* attacks young twigs and growing points where large colonies are often found (Beardsley 1985). It is thought that salivary toxins injected during feeding cause the characteristic distortion of leaves, shortening of internodes and general "bushy-top" symptoms (Dutt et al. 1951, Williams 1996), also known as "tukra leaves" (Veeranna 1997) or "bunchy top" (Singh and Ghosh 1970) associated with the mealybug presence. Additional damage is caused by growth of sooty molds on the honeydew secreted by the feeding insects (Mani 1988).

The impact of the introduction of *M. hirsutus* on the economies of the island countries of the Caribbean has been substantial. Counter-measures have included embargoes of agricultural trade with infested countries, burning of hibiscus plants, and, finally, establishment of long-term biological control programs. Efforts at biological control have focused on three natural enemies: the coccinellids *Cryptolaemus montrouzieri* Mulsant and *Scymnus coccivora* Ramkrisna, and the encyrtid parasitoid *Anagyrus kamali* Moursi (Sagarra and Peterkin 1999). In addition, the USDA-APHIS, PPQ introduced *Gyranusoidea indica* Shaffee, Alam & Agarwal to the U.S. Virgin Islands and Puerto Rico (Anonymous 1997). These biological control agents have been successfully established in the U.S. Virgin Islands (M.S.S., unpublished data) and Puerto Rico, where they have helped to slow the spread of the pest (Michaud and Evans 2000) with the possible help of indigenous predators. However, these programs as well as sanitation and quarantine in the region have been hindered by the lack of an adequate system for detection and population monitoring of *M. hirsutus*. Attraction of males by virgin females has been suspected, based on the biology of nonparthenogenetic

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mealybug species (Aldrich 1996), including the citrus mealybug, *Planococcus citri* (Risso) (Bierl-Leonhardt et al. 1981), and the Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Bierl-Leonhardt et al. 1982). However, male response to female-produced volatiles has not been demonstrated for *M. hirsutus.* Here, we document the ability of virgin female *M. hirsutus* to attract males over a short distance in the laboratory, and at longer distances under field conditions as a first step to identifying a sex pheromone.

Materials and Methods

Insect Rearing. Japanese pumpkins, *Cucurbita moschata* L. 'Chirimen', were grown in the field at the USDA, Agricultural Research Service (ARS), Experimental Station at St. Croix, U.S. Virgin Islands. Monthly plantings of 0.2 ha were made to maintain a permanent supply of mature pumpkins for colony maintenance. Colonies of *M. hirsutus* were established on large Japanese pumpkins (about 1 kg each) from colonies maintained by USDA-APHIS, PPQ at St. Thomas, U.S. Virgin Islands, and later supplemented with field-collected insects from infested areas on St. Croix. Ovisacs were incubated at 28 ± 1°C for 72 h in complete darkness. Age-specific colonies were initiated with crawlers that emerged within a 24-h period. Crawlers were attracted to a small light in a crawler collection box, and transferred with a camel's hair brush onto six to eight large Japanese pumpkins twice per week. Infested pumpkins were maintained in a rearing room at 26 ± 2°C, 70 ± 10% RH, and total darkness.

Two methods were used to produce virgin females: isolation of individual females and elimination of males from colonies by application of sublethal doses of an insect growth regulator (IGR). To exclude male access to individual females, the tips of the long side of No. 1 gelatin capsules (Frontier Natural Products Cooperative, Norway, IA) were cut with a razor blade. The remaining cylinder was carefully glued to large (1-kg) pumpkins, *Cucurbita pepo* L. 'Clipeata'. The smooth skin of these pumpkins and their large size (>30 cm diameter) were preferred over the wrinkled Japanese pumpkins for ease of attaching a larger number of gelatin capsules. Twenty-four or 48 h after gluing the gelatin capsules, a second-instar female nymph was placed in each capsule and capped. Nymphs isolated in gelatin capsules were left in a growth chamber in the dark at 26 ± 2°C and 70% RH until emergence as adult females. An approximately equal number of second-instar female nymphs were freely placed on control pumpkins without gelatin capsules under the same environmental conditions. After the final molt, control females were exposed to 6-h-old males obtained from the colony. Twice as many males as females were released in these control cages. Isolated females in the gelatin capsules were considered mature virgins ≈ 1 wk later, when ovisac production by control females was observed.

Chemical exclusion of males from entire colonies was also used to produce large numbers of virgin females (Bierl-Leonhardt et al. 1981, 1982). Japanese pumpkins were infested with 24-h-old crawlers, as described above. They were placed in an incubator at 27 ± 1°C, 70% RH, and total darkness. Approximately 1 wk later, after the first molt, infested pumpkins were completely submerged for 30–40 s in a 100-ppm solution of the IGR pyriproxifen (Distance, Valent USA, Walnut Creek, CA). After air-drying for 1 h on a laboratory bench, dipped pumpkins were placed in male exclusion cages consisting of a 30 by 30 by 60-cm aluminum frame with silicone-sealed edges and corners, covered with "No-Thrips Insect Screen" (BioQuip, Gardena CA). Cages were kept in a rearing room at 26 ± 2°C, 60 ± 10% RH, and complete darkness. Control pumpkins infested in the same manner but not dipped were kept under the same environmental conditions in a separate rearing room. When one-half of the females on the control pumpkins produced ovisacs, those on the treated pumpkins were considered mature virgin females. If ovisacs were observed, a dipped pumpkin was considered contaminated and rejected for experimental purposes.

Short Range Attraction in the Laboratory. The attractiveness of females to males at a short distance was studied in a laboratory experiment comparing virgins isolated in gelatin capsules to blank controls. About 200 gelatin capsules were glued on each of three large pumpkins. Second-instar female nymphs from age-specific colonies were individually confined in two-thirds of the gelatin capsules on each pumpkin. These capsules were immediately capped to isolate the females. The remaining one-third of the gelatin capsules were kept empty and capped (blank controls). When nymphs in the gelatin capsules reached the adult stage, 60 confirmed virgin females were left on each pumpkin. Thirty blank controls were also maintained on each pumpkin. Additionally, three control pumpkins were infested with ≈600 second-instar female nymphs from the same age-specific colonies. These were maintained in a separate rearing room under the same environmental conditions and continuously exposed to males. Control females were observed periodically to compare their development with that of females in gelatin capsules.

On the day of the experiment, the three pumpkins (replicates) with gelatin capsules were placed on a bench in the center of a 5 by 7-m (105-m^3) laboratory. On each pumpkin, the upper cover of 30 randomly selected gelatin capsules containing one virgin female was manually removed. The other 30 gelatin capsules containing virgin females remained capped for the duration of the experiment. Thirty blanks were also uncapped on each pumpkin, as controls for random settling of males. Approximately 300 males were carefully collected from age-specific colonies within 6 h of emergence using pipette aspirators. Pipettes containing ≈50 males each were left open on the corners of the laboratory to release the males not more than 2–3 m from the pumpkins. The capsules remained open overnight exposed to males for 18 h. The following morning all the open capsules were quickly capped to capture and count any males still inside the gelatin

capsules. All pumpkins were then returned to the rearing room for incubation. Females in the gelatin capsules were observed daily until the presence of ovisacs was noted, and the preoviposition time was recorded. The laboratory was maintained at 25 ± 2°C, 70 ± 10% RH, in the dark, except for the periods of setting up the experiment, releasing the males, and making the counts. Means for males found in capsules were analyzed by analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) test (SAS Institute 1999).

After the preoviposition period, 100 ovisacs from free-living control females were individually placed in gelatin capsules to compare their development and size with ovisacs produced by confined females after their exposure to males. Ovisacs were incubated in the dark at 27 ± 2°C and 70 ± 10% RH. Seven days after eclosion was first observed all gelatin capsules were placed in a freezer at −15°C for 24 h. All crawlers and unhatched eggs were counted for each ovisac. The preoviposition and incubation time, as well as the number of eggs per ovisac, number of crawlers that emerged per ovisac, and percentage of emergence for each ovisac were compared among confined ($n = 83$) and control females ($n = 100$), using Student *t*-test for unpaired samples. Percentages were transformed (arcsine) to stabilize the variance.

Attraction of Males in the Field. To observe if virgin females attracted males under field conditions, traps baited with three densities of virgin females were compared with controls in a completely randomized block design. Traps consisted of white plastic cups (11 by 7 cm) containing a specified number of virgin females and covered with a fine-mesh organdy cloth attached with a tightly stretched rubber band (Meyerdirk and Newell 1979). A white sticky card (7 by 9 cm, Olson Products, Medina, OH) was hung from the rubber band using paper clips. Staples were used to maintain the sticky card in vertical position, perpendicular to the cup's opening, to expose the sticky material on both sides of the card. Traps were baited with 10, 20, or 30 virgin females (7 d old) obtained from dipped pumpkins as previously described. Control traps were identical but did not contain virgin females. Three replicates (traps) for each bait level (number of virgins per trap) were placed directly on hibiscus bushes at three localities on St. Croix, during March and April 1999. Traps were hung from randomly selected plants at ≈1.5 m from the ground. Three localities, LA, Grange (17.7193° N, 64.8786° W; elevation 21 m), Bonne Esperance (17.7436° N, 64.7689° W; elevation 50 m), and Cathrines Rest (17.7256° N, 64.7515° W; elevation 30 m) were used. They all had at least one large hibiscus hedge (>10 m long) with a minimum of 20 mature hibiscus plants naturally infested with *M. hirsutus.* These hedges were planted within a 12-mo period between 1994 and 1995, according to the owners. The mean number of male *M. hirsutus* captured on each trap was analyzed by one-way ANOVA followed by Tukey HSD test. Data for males were transformed to logarithm to stabilize the variance (SAS Institute 1999).

The population density of other *M. hirsutus* stages was estimated at each site. Three terminals ≈20 cm long were randomly selected and cut from hibiscus bushes, brought to the laboratory, and examined under the microscope. Stages of *M. hirsutus* were classified as "egg masses" (ovisacs and crawlers), "females" (second instar to adult), or male pupae for counting purposes. These data were analyzed by ANOVA and Tukey HSD test (SAS Institute 1999).

Long Range Attraction. Baited and unbaited sticky traps 23 by 28 cm (Pherocom, AM Trap, Trécé, Salinas, CA) were placed at several chosen distances from a group of large hibiscus plants (≈2 m tall) at the USDA-ARS Experimental Station on St. Croix. These plants, found in a 6-m^2 area had been naturally infested with *M. hirsutus* for several months. No other hibiscus plants or other infested natural hosts were found within a 100-m radius of the infested plants. Except for two trees found at 25 and 40 m from the infested hibiscus plants, no other physical barriers were present within a 50-m radius from the source of infestation. Sticky traps were baited with 20 virgin females each, placed in a 1.5-cm-diameter glass vial with a perforated cap covered with fine-mesh organdy cloth. Vials were inserted in the lower right corner of the trap. Controls consisted of sticky traps with empty vials. Six traps, three baited and three unbaited, were placed directly on hibiscus plants (0 m) and at 10, 20, 30, and 50 m from the infested plants. Pairs of baited-unbaited traps were spaced a minimum of 20 m from each other, except for those placed directly at the source of infestation (0 m) randomly oriented with respect to the north-westerly direction of the prevailing winds. Traps were left in the field for 72 h, after which they were brought to the laboratory and examined under the microscope. The experiment was repeated three times at monthly intervals in July, August, and September 1999. A $3 \times 5 \times 2$ factorial design was used. The number of males per trap was analyzed as described for the previous experiment including all interactions. Comparisons between baited and unbaited traps were also made at each distance by paired *t*-tests.

Each month, a sample of three terminals (each 20 cm long) was brought to the laboratory; other stages of the mealybug were counted and data were analyzed as previously described.

Results

Insect Rearing. By carefully selecting the age of the crawlers for infesting, colonies produced age-specific insects molting within a 2- to 3-d range. Japanese pumpkins produced between 1,500 and 3,000 females per pumpkin per generation in regular colonies. Pumpkins dipped in the IGR solution produced between 700 and 1,500 virgin females per small (300–600 g) Japanese pumpkin. Although not quantified, mortality was observed in the dipped pumpkins, mostly because of mealybugs falling off the pumpkin during dipping. Male nymphs were able to develop to the "pupal" stage but usually did not complete the last molt

Table 1. Mean ± SEM number of male *M. hirsutus* attracted to virgin females in open or closed gelatin capsules (n = 90 in each treatment), the resulting total of fecund females, and pre-oviposition period after 18 h exposure to males in a dark room

Capsule	Contents	Males per capsule	Total females with ovisac	Pre-oviposition time, d[a]
Open	Virgin	0.97 ± 0.04a	79	3.6 ± 0.6a
Closed	Virgin	0.0 ± 0.00b	4	4.0 ± 0.0a
Open	Empty	0.01 ± 0.01b	—	—

Means followed by the same letter were not significantly different at $\alpha = 0.05$ Tukey HSD test. $F = 118.46$; df = 8, 261; $P < 0.01$.
[a] $F = 1.42$; df = 4, 78; $P < 0.24$.

to adult males. Most male nymphs died either as third instars or as pupae. By careful manipulation after dipping, and by keeping treated pumpkins in cages in a separate rearing room, contamination by males was avoided. Females remained virgin (no ovisacs observed) in these cages for more than 30 d.

Short Range Attraction in the Laboratory. More males were found in the open gelatin capsules containing virgin females than on the empty, uncapped ones (Table 1). A single male was found in one open gelatin capsule without a virgin female, whereas 87 males were found in open gelatin capsules containing virgin females. Most of the latter capsules (83.3%) contained one male and 6.7% contained two males. Of those females in the gelatin capsules that attracted at least one male, 79 (97.5%) successfully produced an ovisac, an indication that mating occurred. Four ovisacs were found in four gelatin capsules that were never opened during the experiment. The preoviposition period for confined females was similar to that of free-living females but ovisacs from confined females took about one-half day longer to incubate (Table 2). The ovisacs from confined females had fewer eggs and produced fewer crawlers than those from free-living females; however, the percentage of eclosion was not different (Table 2).

Attraction of Males in the Field. The number of males found on sticky traps was affected by the number of virgin females used as bait and by the locality. There was no interaction between these factors. Traps containing 20 virgin females attracted 58% more males than traps with 10 females (Table 3). Traps with 30 virgin females did not catch significantly more males than traps with 20. Males were also captured on control traps with no virgin females. More males were captured at Cathrines Rest than at Bonne Esperance and La Grange (Table 4). Population density counts on terminals, made simultaneously with the trapping of males showed no statistical differences among the three localities (Table 4). There were between 48 and 76 egg masses, between 337 and 509 females, and between five and 15 male pupae per terminal.

Table 2. Comparison (means ± SEM) of ovisacs from *M. hirsutus* females confined in gelatin capsules (n = 83) and free-living on Japanese pumpkins (n = 100)

Mean per ovisac	Females in capsules	Free-living females	$P > t$[a]
Pre-oviposition, d	3.6 ± 0.1	3.8 ± 0.1	0.08
Incubation, d	6.6 ± 0.1	6.1 ± 0.1	<0.01
No. eggs	124.1 ± 8.2	155.3 ± 8.4	<0.01
Crawlers	111.1 ± 7.6	134.7 ± 8.3	0.04
% eclosion[b]	89.1 ± 0.9	85.5 ± 1.7	0.08

[a] Probability of a greater t statistic for unpaired comparisons, Student t-test.
[b] Percentages were transformed (arcsine). Untransformed means shown.

Table 3. Number (mean ± SEM) of male *M. hirsutus* attracted to sticky traps (n = 9 in each treatment) baited with different numbers of virgin females on St. Croix, U.S. Virgin Islands

No. virgin females per trap	No. males captured per trap
0	4.1 ± 1.4a
10	16.7 ± 4.4ab
20	40.0 ± 9.3c
30	29.0 ± 6.3bc

Means followed by the same letter were not significantly different at $\alpha = 0.05$, Tukey's HSD Test. $F = 7.425$; df = 3, 24; $P < 0.01$.

Long Range Attraction. The distance from the infested plants, the month, and the baiting of traps affected the number of males captured. Baited traps always attracted significantly more males than unbaited traps. There was no significant interaction between month and distance ($F = 0.58$; df = 8, 60; $P = 0.793$), or between distance and level of baiting ($F = 0.88$; df = 4, 60; $P = 0.479$). There was a significant interaction between month and bait ($F = 3.82$; df = 2, 60; $P = 0.028$). The interaction term among all effects, month by distance by baiting was not significant ($F = 0.8$; df = 8, 60; $P = 0.602$).

Significantly larger captures were obtained in July and August than in September 1999 ($F = 43.7$; df = 2, 60; $P < 0.01$). Because there was a significant interaction between month and level of bait, baited and unbaited captures were separately compared on each month. Almost seven times more males were captured by baited than unbaited traps ($F = 154.2$; df = 1, 60; $P < 0.01$) during July and August (Fig. 1) and 4.5 times more in September. Lower captures were obtained in September on both baited and unbaited traps.

Baited traps had maximum captures ($F = 16.2$; df = 4, 60; $P = < 0.01$) at 0 and 10 m from the infested plants (Fig. 2). Fewer males (100–140) were captured at distances >20 m. Virgin females in these traps were able to attract 101.6 ± 27.7 (mean ± SEM) males at 50 m from the infested plants that were considered the sole source of males in this experiment. Unbaited traps captured significantly more males ($F = 6.81$; df = 4, 8; $P < 0.01$) when they were located closer to the source of infestation (0–20 m). Between 11 and 15 males were captured per trap 30 and 50 m away from the source of males. There was a significant ($F = 90.9$; df = 1, 4; $P < 0.01$) logarithmic relationship between the number of males captured by baited traps and the distance from the source of infestation (Fig. 2). The number of

Table 4. Mean number (±SEM) of male *M. hirsutus* captured on sticky traps at three locations on St. Croix, U.S. Virgin Islands, and population density counts on 20-cm terminals during March and April 1999

Locality	No. males per trap	No./Terminal		
		Egg masses[a]	Females[b]	Male pupae[c]
La Grange	13.0 ± 3.9a	48.3 ± 10.1	337.0 ± 197.6	9.7 ± 1.5
Bonne Esperance	20.9 ± 6.3ab	59.3 ± 18.9	422.3 ± 224.9	5.7 ± 2.2
Cathrines Rest	33.4 ± 7.4b	76.0 ± 19.7	509.3 ± 242.9	15.3 ± 6.7

Means followed by the same letter were not significantly different at $\alpha = 0.05$ Tukey HSD test. $F = 4.367$; df = 2, 24; $P = 0.024$.
[a] Included eggs and crawlers. $F = 0.689$; df = 2, 6; $P = 0.538$.
[b] Includes second instar to adult females. $F = 0.15$; df = 2, 6; $P = 0.864$.
[c] $F = 1.303$; df = 2, 6; $P = 0.339$.

males per trap decreased as the distance from the source of the infestation increased. For unbaited traps, a similar relationship ($F = 9.09$; df = 1, 4; $P = 0.06$) was found.

When counts of other life-stages were made simultaneously with male-trappings, approximately six times more egg masses were found per 20-cm terminal in July than in August or September (Table 5). The number of females per terminal was not significantly different during any of the three monthly evaluation periods, ranging from 227 to 900 per terminal. The number of male pupae found on terminals was also not significantly different during any of the three monthly samplings.

Discussion

Most virgin females isolated in gelatin capsules were able to attract at least one male, and most of them mated successfully, as evidenced by the production of fertilized eggs. Confining females in gelatin capsules could have two types of effects. First, it could negatively affect their behavior and render them sexually inactive, although this does not seem to be the case in this experiment. Alternatively, confinement could concentrate volatiles and artificially enhance female attraction of males, especially at short range. We do not know when females began producing and releasing sex pheromones but we can speculate that females started releasing volatiles as soon as they were ready to mate, within a few days before the experiment, as has been shown for other mealybug species (Aldrich 1996). The preoviposition periods found here are similar to previous reports for *M. hirsutus* of 0.5–6 d, (Ghose 1972, Mani, 1989). The preoviposition period and percentage eclosion of eggs were similar between confined and free-living females, suggesting that virgins in the gelatin capsules were not different from control females with respect to maturation rate or fertility. Negative impacts of female confinement might be reflected in the production of smaller ovisacs with longer incubation periods. Confinement, however, did not affect the percent eclosion of ovisacs.

It appears that males were attracted to volatiles produced by virgin females at short distances in the laboratory, instead of flying randomly to find a female, because there were practically no males found in empty gelatin capsules. This does not exclude the possibility of males alighting on empty capsules and "correcting" to find a virgin-containing capsule. This mode of attraction seems to enable virgin females to attract a single male because only in a few cases (6.7%) were two males found in capsules with virgin females. This also suggests that females stop producing pheromones soon after mating as has been reported in other mealybug species (Aldrich 1996).

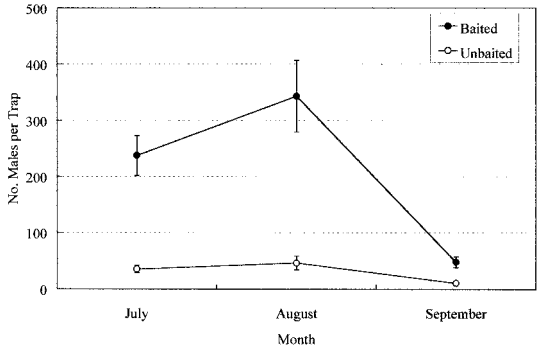

Fig. 1. Mean ± SEM number of *M. hirsutus* males captured on sticky traps baited with 20 virgin females ($n = 15$) or unbaited controls, at monthly intervals at the USDA-ARS Experimental Station on St. Croix, U.S. Virgin Islands.

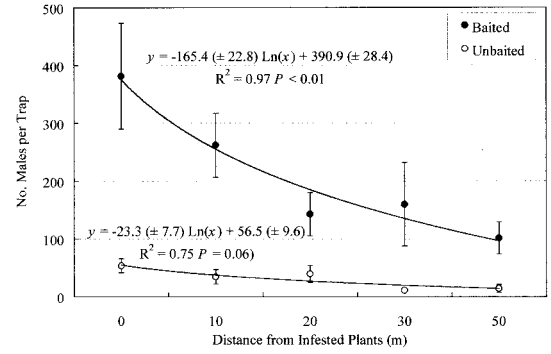

Fig. 2. Relationship between the distance from infested plants and the number of males captured per trap baited with 20 virgin *M. hirsutus* females or unbaited control traps at the USDA-ARS Experimental Station on St. Croix, U.S. Virgin Islands. Error bars are ±SEM ($n = 9$).

Table 5. Mean number (±SEM) of other stages of *M. hirsutus* on 20-cm hibiscus terminals at monthly intervals ($n = 3$) at the USDA, ARS Experimental Station on St. Croix, U.S. Virgin Islands

Month	Stage/20-cm terminal		
	Egg masses	No. females[a]	Male pupae[b]
July	149.7 ± 47.8a	528.7 ± 223.6a	21.7 ± 4.9a
August	26.7 ± 6.7b	900.7 ± 208.9a	27.7 ± 5.9a
September	25.3 ± 5.5b	227.0 ± 45.8a	17.7 ± 2.6a

Means followed by the same letter were not significantly different at $\alpha = 0.05$ by Tukey's HSD test. $F = 15.02$; df = 2, 6; $P < 0.01$.
[a] $F = 3.57$; df = 2, 6; $P = 0.095$.
[b] $F = 3.57$; df = 2, 6; $P = 0.095$.

Even though extreme care was taken when gluing the gelatin capsules, males have been known to somehow find small openings formed as the glue dries, to gain access to females (D.E.M., unpublished data). This would explain the presence of ovisacs in gelatin capsules that were closed during exposure to males. However, because no males were found inside the capsules, mating could also have accidentally occurred before the experiment.

Field trapping of males using virgin females was previously done in California with the Comstock mealybug (*P. comstocki*). Meyerdirk and Newell (1979) maintained virgin females in the field on sprouted potatoes for longer periods than females in our experiments that were not provided with food. Although females in our traps were still alive and active at the end of the 3-d trapping period, we do not know if their attractiveness to males or their production of volatiles declined during this period. Virgin females of the Comstock mealybug can attract males for a period of 1 mo (Meyerdirk and Newell 1979) and nonfeeding females produce an average of 15 ng of pheromone over a 15-d period (Bierl-Leonardt et al. 1982). In other mealybug species, pheromone emission is continuous until copulation takes place (Aldrich 1996).

Volatiles produced by virgin female *M. hirsutus* seem to be most effective at short distances. This was shown in the laboratory, where almost every female attracted a male, and in the field where traps closest to the infested plants captured the most males. As the distance from these plants increased, fewer males were attracted to baited traps. This might suggest a concentration-dependent response by flying males also evidenced by the apparent reduction in captures with >20 females in the baits. Pheromone-mediated attraction of males over long distances has been reported in other mealybug species. Moreno et al. (1972) reported that traps with 10 virgin females attracted males of *P. comstocki* at distances up to 33.5 m. *M. hirsutus* males may also engage in random flights because males were captured in unbaited traps, even at long distances. Considering that the life span of males is ≈1–2 d (Ghose 1972), a sex pheromone-mediated system would be expected to recruit randomly flying males from long distances, but it also would allow males to fly away from their original clusters (i.e., at higher concentrations) to avoid inbreeding. This could be further investigated using larger numbers of virgin females as bait, to test the slight reduction in male captures on traps baited with 30 virgin females. Long distance recruitment of males could be an advantage for insects with patchy distributions when population densities are low. A sex pheromone (or pheromones) that works in such a system would probably diffuse slowly in the air, allowing for long-term or long-distance recruitment of males. Research is underway to isolate and synthesize a pheromone that could be used for baiting traps.

A relationship between the number of males and the density of other stages in the population has been difficult to establish. Meyerdirk et al. (1981) found no correlation between the numbers of male *P. comstocki* attracted to 10 or more virgin females in pheromone traps and the relative population density on leaves of mulberry trees (*Morus alba* L.). A long-term program for sampling *M. hirsutus* populations on these localities using sticky traps with virgin females and density counts on terminals has been initiated on St. Croix to further investigate these relationships.

Our results suggest that virgin-female *M. hirsutus* use sex pheromones to attract males for mating and that these volatiles can attract males from up to 50 m away from infested trees or further. Until a pheromone is identified and synthesized, virgin females can be used in sticky traps to attract random flying males for population monitoring. The introduction of the *M. hirsutus* into the U.S. mainland increases the urgency of identifying and synthesizing these volatiles for monitoring and estimating population densities.

Acknowledgments

We thank Esther Peregrine and Jose Gonzales (USDA-ARS, GIRU St. Croix) for supplying most of the Japanese pumpkins used in this study. Richard Warkentin (USDA-APHIS, PPQ St. Thomas, U.S. Virgin Islands) supplied mealybugs and advice for field population counts. Henry Schuster (Commissioner, U.S. Virgin Islands Ministry of Agriculture) supplied pumpkins used in laboratory experiments. Heidy von Ryan (Estate Bonne Esperance), the Harvey family (Estate Cathrines Rest), and Oscar Henry (Estate La Grange) kindly authorized the placement of traps in their properties on St. Croix. Mike Riffle (Valent USA Corporation) supplied a sample of Distance for our experiments. We are also grateful to two anonymous reviewers whose comments and suggestions greatly improved the manuscript.

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Received for publication 9 June 2000; accepted 22 November 2000.